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Efficacy of afoxolaner against *Ixodes scapularis* ticks in dogs

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ABSTRACT

Efficacy of afoxolaner, a novel isoxazoline insecticide/acaricide, against *Ixodes scapularis* was evaluated in a laboratory study. One day prior to treatment, beagle dogs ($n = 16$) were infested with 50 unfed wild adult ticks. Repeat infestations were performed weekly for four additional weeks. The number of live ticks remaining on each dog was determined 48 h after treatment and after each subsequent infestation. A single oral treatment with a dose approaching the minimum effective dose of afoxolaner (2.5 mg/kg) eliminated the pre-existing infestations of *I. scapularis* ticks and controlled weekly re-infestations, with efficacy between 98% and 100% recorded until Day 23 and 94% at Day 30.

1. Introduction

Tick control is an important concern for public health officials, pet owners, and veterinarians (Dantas-Torres et al., 2012; Mencke, 2013). *Ixodes scapularis* ticks have become an increasingly important concern for public and veterinary health (Dantas-Torres et al., 2012; Otranto and Wall, 2008). These ticks can carry a variety of infectious agents, some of which are zoonotic and may be life-threatening, including the organisms that cause Lyme borreliosis, babesiosis, and ehrlichiosis in both dogs and humans (Chomel, 2011; Colwell et al., 2011; Varde et al., 1998). The geographic distribution of *I. scapularis* ticks is also expanding, in part through infestation of migratory birds (Hamer et al., 2012; Ogden et al., 2008). Several tick species, including *I. scapularis*, are also capable of transmitting a salivary neurotoxin that can block acetylcholine transmission and lead to flaccid paralysis in dogs and humans (Blagburn and Dryden, 2009; Vedanarayanan et al., 2004).

There are several strategies available to control tick infestations, including avoidance of infested environments, particularly during periods when ticks are active (Otranto et al., 2009; Blagburn and Dryden, 2009). Regular administration of an acaricide is also important since owners may not be aware of what tick species are common in their area and avoiding infested environments may be difficult. There are many topically applied acaricidal products currently available as spot-on formulations or collars. These compounds, which can be highly efficacious against some tick species, include amitraz, fipronil and pyrethroids (permethrin, deltamethrin, and flumethrin) (Beugnet and Franc, 2012). Despite their reported effectiveness, there are some concerns about the use of topical products due to differences in dogs' hair coats that may affect efficacy, adverse effects of shampooing or bathing after application on efficacy, possible toxic effects of products on non-target species, or cosmetic concerns (Dryden and Payne, 2004; Malik et al., 2010). Therefore, an orally administered acaricide may be preferable for many pet owners.

Afoxolaner is a novel insecticide–acaricide administered orally in a chewable formulation (Nexgard®, Merial) designed to treat and control fleas and ticks on dogs. This paper describes an experimental study that was performed

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to demonstrate the efficacy of afoxolaner against *I. scapularis*, a North American tick species that commonly infests dogs.

2. Materials and methods

2.1. Experimental design

A study was conducted to demonstrate the efficacy of afoxolaner against *I. scapularis*. The study was performed in the United States and was designed in accordance with standard methods for evaluating the efficacy of parasiticides for the treatment, prevention and control of tick infestations (Marchiondo et al., 2013). It complied with Merial and local Institutional Animal Care and Use Committee requirements and international laws and ethics standards. Dogs were managed with regard to US Animal Welfare Regulations, 2008, 9 CFR (USDA, 2008).

2.2. Animals

The study involved 16 purpose bred beagles, which were individually identified by unique ear tattoos. Eight male and eight female dogs aged 6–8 months and weighing 5.2–8.7 kg were included. Dogs were in good health and had not been treated with ectoparasiticides for at least 3 months prior to the start of the study. Tick infestations and subsequent counts were performed prior to treatment and confirmed that the dogs were capable of maintaining adequate tick infestations. Dogs were housed individually. Health observations were conducted daily throughout the study. In addition, health observations were conducted every hour for 4 h following treatment with afoxolaner on Day 0.

2.3. Study design

The study followed a controlled, randomized block design, including 16 dogs (8 per group). Six days prior to treatment dogs were infested with 50 adult *I. scapularis* ticks, which were removed and counted 48 h later. The pre-treatment tick counts were used to allocate dogs to either the control or afoxolaner-treated group. Dogs in Group 1 were untreated controls. Dogs in Group 2 were treated once orally on Day 0 with the appropriate combination of soft chewables containing afoxolaner. Chews weighing 0.5 g and containing 11.3 mg of afoxolaner were used. As the chewables are not designed to be divided, the dosing was administered as closely as possible to the minimum effective dose of 2.5 mg/kg using whole chews. The actual dosage administered to the dogs ranged from 2.7 to 3.7 mg/kg of body weight.

Dogs were infested with 50 adult ticks on the day prior to treatment (Day −1) and on Days 7, 14, 21, and 28. Forty-eight hours after treatment and 48 h after each of the subsequent re-infestations, ticks were removed and live ticks counted. These counts were conducted during a process that involved methodical examination of all body areas using finger tips and/or a coarse tooth comb to sort through the hair and locate all ticks on the animal, as described by Marchiondo et al. (2013). The *I. scapularis* ticks used in this study were unfed adult ticks, approximately 50% male and 50% female, collected from the wild in the USA. Personnel responsible for collection of animal health and efficacy data were blinded to the treatment groups.

Table 1
Geometric mean (range) live *Ixodes scapularis* tick counts on control dogs 48 h after treatment (Day −1 infestation) or after subsequent re-infestations and percent efficacy on treated dogs.

Day of infestation	Day of tick count	Geometric mean live ticks (range)[a]	% Efficacy
−1	2	21.1 (15–29)	98.4
7	9	14.1 (4–25)	100
14	16	20.9 (15–32)	99.1
21	23	20.9 (15–29)	99.6
28	30	14.2 (7–29)	94.2

[a] There was a significant difference ($p < 0.001$) in tick counts between afoxolaner-treated and control dogs at all time points up to Day 30.

2.4. Data analysis

Total counts of live ticks were transformed to the natural logarithm of (count + 1) for calculation of geometric means by treatment group at each time point. Percent reduction from the control group mean was calculated for the treated group at each post-treatment time point using the formula $[(C - T)/C] \times 100$, where C is the geometric mean for the control group and T is the geometric mean for the treated group. The log counts of the treated group were compared to the log counts of the untreated control group using an F-test adjusted for the allocation blocks used to randomize the animals to the treatment groups. The mixed procedure in SAS® version 9.1.3 was used for the analysis, with treatment group listed as a fixed effect and the allocation blocks listed as a random effect. The comparisons were performed using a two-sided test with a 5% significance level.

3. Results

No vomiting was reported in treated dogs during the study based on observations every hour for 4 h after dosage and daily observations thereafter. No treatment-related health problems were observed throughout the study. The number of ticks counted on untreated control dogs exceeded 20% of the challenge (50 ticks) at all time points, as recommended by Marchiondo et al. (2013), to allow a robust comparison with the treated dogs (Table 1).

Efficacy results against *I. scapularis* are presented in Table 1. Dogs were infested one day prior to treatment and afoxolaner provided 98.4% curative efficacy against *I. scapularis* at the 48 h count (Table 1). Dogs were re-infested with 50 adult ticks on a weekly basis through Day 28, and the efficacy 48 h after each re-infestation was 100%, 99.1%, 99.6%, and 94.2% at Days 9, 16, 23, and 30, respectively (Table 1). There was a significant difference ($p < 0.001$) between treated and control dogs for counts of ticks at all time points through Day 30 (Table 1).

4. Discussion

In this study a single oral dose of afoxolaner at the minimum effective dose was highly effective in eliminating existing infestations of *I. scapularis*, with efficacy of 98.4% within 2 days following treatment. It also provided extended efficacy following re-infestation with ticks, with >99% up to 23 days after treatment and >94% efficacy one month after treatment.

Nexgard® is the first orally administered product that kills *I. scapularis* ticks. Afoxolaner acts systemically and requires ticks to feed, however, the study reported here demonstrated persistently high efficacy assessed at 48 h. Such efficacy is similar to other commercialized products that are applied topically. For example, one study assessed the efficacy of topical products containing fipronil + (S)-methoprene and imidacloprid + permethrin against *I. scapularis* ticks; results were 98.4% and 96.5%, respectively, 48 h following infestation on Day 30 (Dryden et al., 2006). Concern has been expressed that systemically acting products would take longer to kill ticks than topically applied products. For instance, Marchiondo et al. (2013) suggest that tick attachment of 72 h or longer may be acceptable for products with a systemic mode of action. However, the results from the current study demonstrate that afoxolaner, administered orally in Nexgard®, provides >90% efficacy against *I. scapularis* within 48 h of infestation for at least one month.

It is important that products used to control tick infestations be effective at a level greater than 90%, in order to provide relief from blood loss and irritation associated with tick bites (Marchiondo et al., 2013; Dryden and Payne, 2004).

The oral route of administration may be preferred by some owners over topical parasiticide products. For instance, the efficacy of some topical products may be affected by bathing or swimming, or there may be a period of time in which the application site should be avoided by pet owners or other household animals (Dryden and Payne, 2004). Also, the density and length of the dog's hair coat does not affect the application of an oral product as it can with a topical product. The chewable formulation of Nexgard® is also advantageous in that it is palatable and voluntarily consumed by dogs, making it easy and convenient for owners to administer.

5. Conclusions

This study demonstrated the efficacy of a single oral dose of afoxolaner against *I. scapularis*. Existing tick infestations were rapidly cleared and there was a residual protection against ticks for at least a month.

Conflict of interest

The work reported herein was funded by Merial Limited, GA, USA. All authors are current employees or contractors of Merial.

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